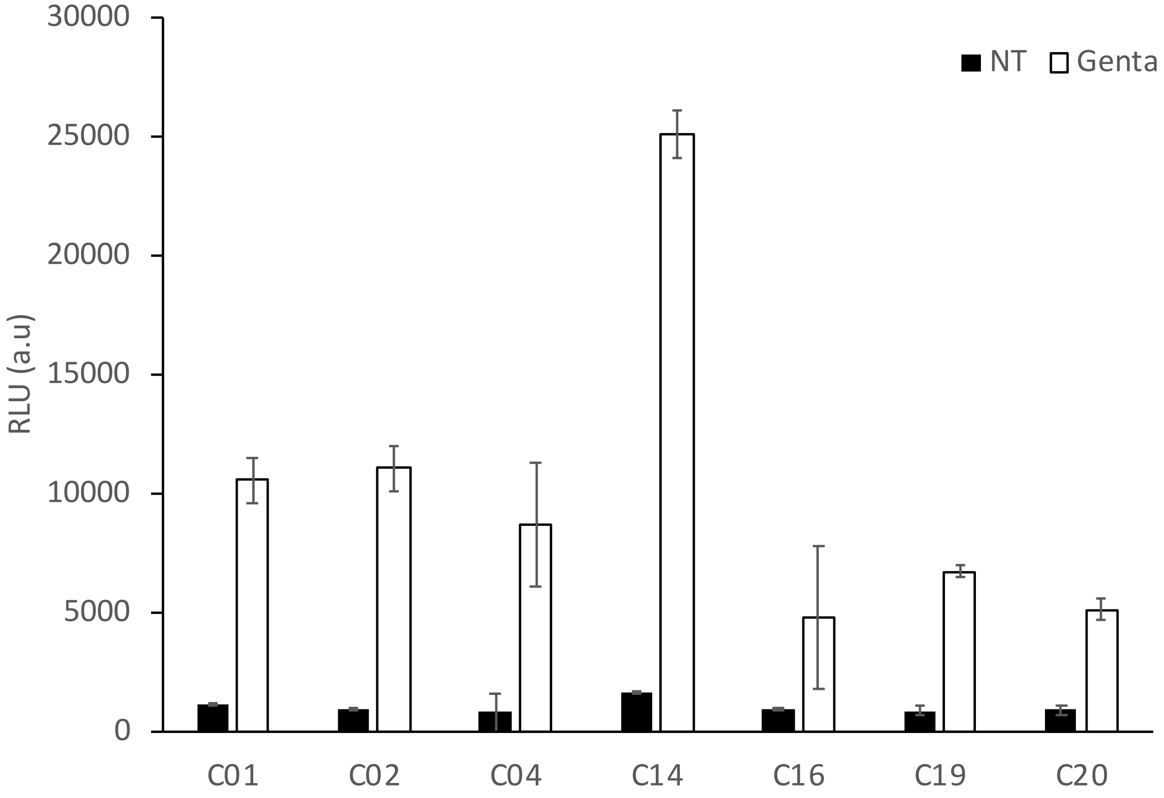

Figure S1. Selection of the recombinant cell line for HTS

Recombinant NIH3T3 cells were cultured in 96-well plates, at an initial density of 15,000 cells/well. Luciferase activity was quantified by adding coelenterazine directly to the wells (*n*=4). Black and white bars represent basal *Metridia* luciferase activity and gentamicin (1.6 mM)-induced activity, respectively. We retained clone number C14 for further development.

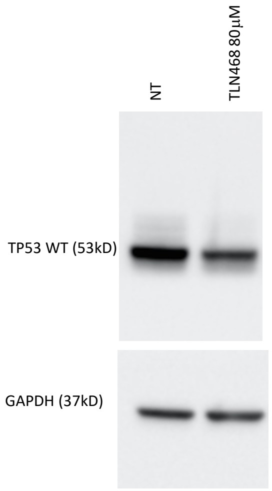

Figure S2. **WT TP53 is not stabilized by TLN468.** Total proteins were extracted from H1299 cells, expressing the WT TP53, treated or not (NT) with TLN468. GAPDH is used as a loading control.

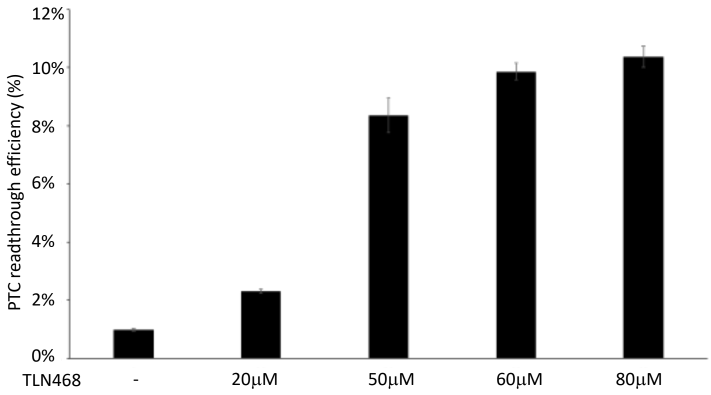

Figure S3. TLN468 dose-response. Hela cells were transfected with pAC99-R213X reporter system and treated with increasing concentration of TLN468 (from 0 to 80μM) during 24H. Bars represent the median value of the ratio between R213X and the in-frame control. At least four independent experiments for each treatment have been performed.

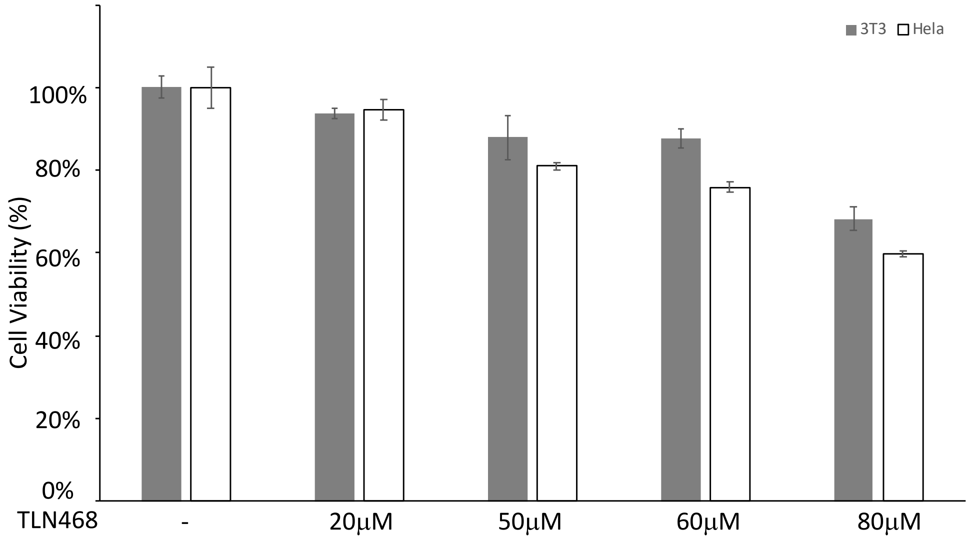

Figure S4. TLN468 toxicity assay. Hela and NIH3T3 cells were treated or not with different doses of TLN468 during 24 hours. Viability was quantified after addition of a tetrazolium salt wich is cleaved to formazan only in viable metabolically active cells.

Figure S5. TLN468 does not impact the level of NMD-sensitive transcripts. Venn Diagramm comparing the 286 human genes described by Lou C.H *et al*. Stem Cell rep; 6, 6; 844-857 (2016) to be sensitive to NMD and stabilized by deletion of UPF1 (Blue, NMD), with genes for which we observed an increase (UP) or a decrease (DOWN) of RNA levels by RNAseq in presence of TLN468 (Log2FC ≤ -1 or Log2FC ≥1 with a padj < 10^{-3}).

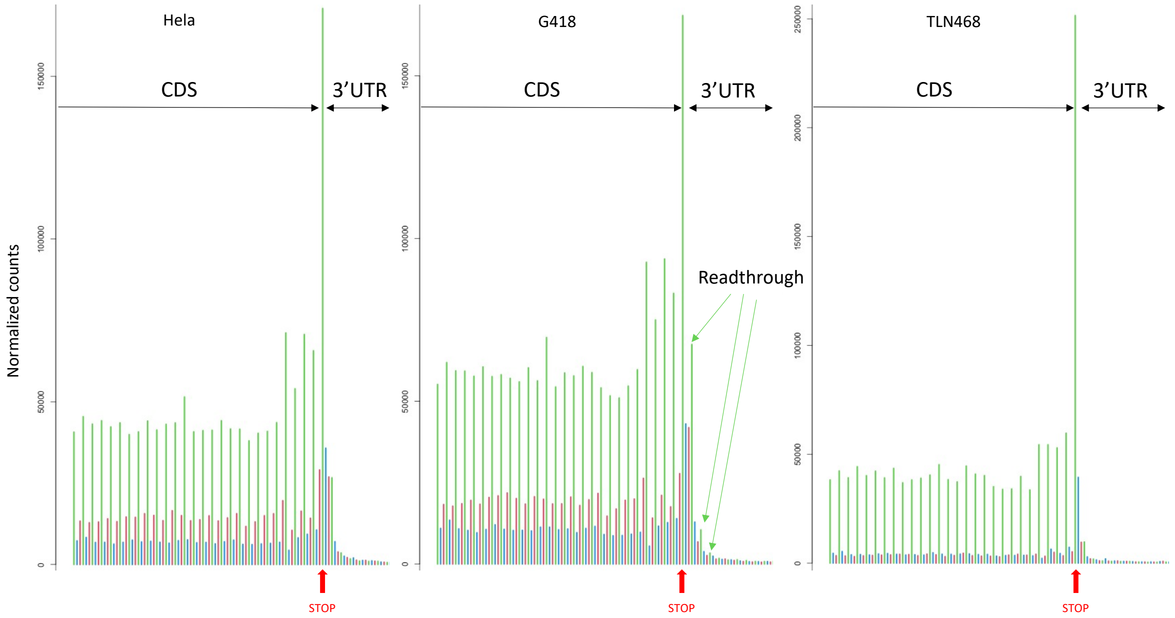

Figure S6. Metagene of RiboSeq. Metagene representation of RiboSeq periodicity for 29-mers RPF of the three samples (Hela, G418 and TLN468). Each bar represents the average value for the A-site position of each RPF for three independent experiments normalized to the size of the library. The red arrow indicates the position of the first nucleotide of the stop codon. Frames 0, +1 and +2 are indicated in green, blue and red respectively. Y-Axis scale has been adapted to fit the accumulation of ribosomes at the TC.

Name	Nonsense mutation	Frequency (%)	% RT DMSO	SD	% RT Genta	SD	%RT TLN468	SD
R145X	AGC TGG GTC TGA CAA TCA ACT	0.75	1.73%	0.10%	3.10%	0.25%	3.24%	0.54%
S147X	GTC CGA CAA TGA ACT CGT AAT	0.14	0.17%	0.05%	0.38%	0.04%	0.55%	0.07%
Q194X	TCA GCC ACA TAA CGA CTG GAA	2.63	0.04%	0.00%	0.05%	0.01%	0.12%	0.01%
R195X	GCC ACA CAA TGA CTG GAA CAT	0.61	1.76%	0.32%	2.50%	0.37%	1.16%	0.21%
Q267X	GAA CAT TTT TAG TTA CAT CAT	2.63	0.03%	0.00%	0.02%	0.01%	0.16%	0.16%
R539X	TTG GGA GAT TGA TGG GCA AAC	0.34	0.07%	0.01%	0.64%	0.08%	0.22%	0.05%
Q555X	GTT CTT TTA TAA GAC ATC CTT	0.07	0.02%	0.00%	0.06%	0.01%	0.06%	0.02%
L654X	TGG GAT ATT TAA CAT CAA AAA	0.07	0.02%	0.00%	0.04%	0.00%	0.03%	0.01%
E761X	GAC TTA AAA TAA AAA GTC AAT	0.55	0.02%	0.00%	0.02%	0.00%	0.11%	0.01%
R768X	GCC ATA GAG TGA GAA AAA GCT	0.82	0.06%	0.01%	0.08%	0.01%	0.52%	0.13%
R1051X	AAT AAA CTC TGA AAA ATT CAG	0.55	0.10%	0.01%	0.13%	0.01%	1.68%	0.14%
W1075	AAG GAG GAA TAG CCT GCC CTT	2.63	0.05%	0.01%	0.09%	0.03%	0.19%	0.02%
E1182X	GAG TAT CTT TAG AGA GAT TTT	0.07	0.02%	0.00%	0.03%	0.00%	0.35%	0.03%
W1268X	TGG GCA TGT TGA CAT GAG TTA	0.07	0.20%	0.02%	0.71%	0.09%	2.85%	0.63%
R1577X	CGT AAG ATG TGA AAG GAA ATG	0.68	0.28%	0.02%	0.32%	0.06%	0.95%	0.20%
R1666X	GTC ACC TCC TGA GCA GAA GAG	0.75	0.06%	0.00%	0.11%	0.01%	0.45%	0.05%
R1844X	GAG AGA AAG TGA GAG GAA ATA	0.27	0.04%	0.01%	0.12%	0.02%	0.25%	0.05%
R1868X	AGG TCT CAA TGA AGA AAA AAG	0.61	0.02%	0.00%	0.03%	0.01%	0.17%	0.03%
W1879X	TCT CAT CAG TGA TAT CAG TAC	0.07	0.08%	0.01%	0.13%	0.03%	1.17%	0.13%
Y1882X	TGG TAT CAG TAA AAG AGG CAG	0.14	0.01%	0.00%	0.02%	0.00%	0.03%	0.00%
W1956X	AGC AAG CGC TAG CGG GAA ATT	0.07	0.02%	0.00%	0.07%	0.00%	0.09%	0.01%
R1967X	GCT CAG TTT TGA AGA CTC AAC	0.61	0.06%	0.00%	0.35%	0.05%	0.63%	0.05%
E2035X	TTT AAG CAA TAG GAG TCT CTG	5.26	0.10%	0.01%	0.12%	0.02%	0.18%	0.04%
R2095X	TAC AAG GAC TGA CAA GGG CGA	0.55	0.33%	0.04%	0.60%	0.04%	1.05%	0.08%
E2286X	ATA AGC CCA TAA GAG CAA GAT	2.63	0.01%	0.00%	0.01%	0.00%	0.12%	0.01%
Q2526X	AGG CGT CCC TAG TTG GAA GAA	0.07	0.04%	0.00%	0.11%	0.01%	0.40%	0.04%
R2553X	ATT ACG GAT TGA ATT GAA AGA	0.82	0.18%	0.01%	0.35%	0.02%	0.84%	0.05%
Q2574X	CGG AGG CAA TAG TTG AAT GAA	2.63	0.03%	0.00%	0.05%	0.00%	0.13%	0.03%
K2791X	GAA CTT CGG TAA AAG TCT CTC	2.63	0.02%	0.00%	0.02%	0.00%	0.12%	0.01%
R2870X	GAG ACT GTA TGA ATA TTT CTG	0.82	0.06%	0.00%	0.08%	0.01%	0.69%	0.11%
R2905X	CGG CTT CTA TGA AAG CAG GCT	0.34	0.11%	0.02%	0.16%	0.02%	3.38%	0.54%
W2925X	TCC GCT GAC TGA CAG AGA AAA	0.14	0.15%	0.03%	0.95%	0.21%	0.70%	0.14%
R2982X	AAG GCA CTT TGA GAA GAA AAT	0.55	0.05%	0.00%	0.15%	0.03%	0.71%	0.07%
R3034X	GTC GAG GAC TGA GTC GTC CAG	0.55	0.06%	0.01%	0.09%	0.01%	0.51%	0.12%
S3127X	TTG AGC CTG TGA GCT GCA TGT	2.63	0.02%	0.00%	0.03%	0.00%	0.17%	0.02%

R3190X	GAT ACG GGA TGA ACA GGG AGG	0.89	0.19%	0.01%	0.24%	0.03%	1.92%	0.39%
R3345X	TTT TCT GGT TGA GTT GCA AAA	0.48	0.04%	0.00%	0.01%	0.00%	0.10%	0.01%
R3370X	GAA GAT GTT TGA GAC TTT GCC	1.02	0.04%	0.00%	0.10%	0.02%	0.46%	0.10%
R3381X	AAC AAA TTT TGA ACC AAA AGG	1.57	0.05%	0.01%	0.19%	0.03%	0.29%	0.08%
R3391X	AAG CAT CCC TGA ATG GGC TAC	1.5	0.13%	0.01%	0.25%	0.03%	0.27%	0.01%

Table S1. List of the 40 more frequent PTC found in DMD